SOME RESEARCHES ON THE LIFE-CYCLE OF SPIROCHAETES

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(*Received for publication* 9 *November*, 1911)

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INTRODUCTION

The elucidation of the complete life-cycle of Spirochaetes is a matter of considerable importance from the scientific and economic point of view. African tick fever and European relapsing fever are due to *Spirochaeta duttoni* and *S. recurrentis* respectively, while *S. marchouxi** has fatal effects in chickens. Spirochaetes may also occur in the digestive tract of many hosts, as is the case in many game birds and in a large number of molluscs. Both blood-inhabiting Spirochaetes and those of Lamellibranchs have claimed my attention for some years, and the following are notes supplementing my previous work, and contributing new items to our knowledge of the life-history of Spirochaetes.

MATERIAL AND METHODS

The work relates to Spirochaetes of the blood, *S. duttoni*, *S. recurrentis* and *S. marchouxi* (= *gallinarum*),* and comparison has been made throughout with the Spirochaetes of Lamellibranchs,

* The Spirochaete of fowls, first described by Marchoux and Salimbeni in 1903, was named *S. marchouxi* by Nuttall in a paper read on Dec. 9, 1904. It was also named *S. gallinarum* by Stephens and Christophers in a book with a preface dated Nov., 1904, but published in 1905. References are given on p. 496.

S. balbianii in *Ostrea edulis* and *Tapes aureus*, *S. anodontae* and *S. solenis* (nov. sp., with pointed ends) from *Solen ensis*.

Many observations have in each case been made on the living organisms, and confirmed later by the examination of stained preparations. For examination of fresh material, use has been made of thermostats and warm stages kept at 37° C. and at 25° C., while preparations have also been examined at room temperature. The paraboloid condenser has been of service throughout, though not indispensable. For staining, iron haematoxylin, Delafield's haematoxylin, gentian violet, thionin and Giemsa's stain have been of most use after wet fixation with osmic acid, corrosive acetic alcohol or Bouin's fluid. Zeiss 1/12″ and 2 mm. objectives with compensating oculars 8 and 12 have been used.

BLOOD-INHABITING SPIROCHAETES

Spirochaeta duttoni, *S. recurrentis* and *S. marchouxi*

These Spirochaetes have long, narrow bodies with many spiral coils. Each has a firm cuticle or periplast from which the protoplasmic

Fig. 1.—Diagram of *S. duttoni*, showing chromatin granules, pointed ends and slight membrane edge.

contents can be squeezed out with much difficulty, leaving the empty periplastic sheath or cuticle behind, as was pointed out by Stephens in 1906. A very tenuous membrane is present (Fig. 1),

being often so closely contracted against the body that it is almost invisible in the living organism and in many stained specimens. The nucleus consists of a series of bars or rodlets ('granules') of chromatin distributed along the body. The structure is most difficult of discernment owing to the minute diameter of the body, but after prolonged staining with Romanowsky solution the body exhibits alternate red areas of chromatin and paler bluish areas of cytoplasm. In life the body appears homogeneous, probably owing to the refractivity of the periplast.

The figures of the structure of *S. marchouxi*, published by Prowazek in 1906, seem to me to be most accurate.

DIVISION

In the case of the above Spirochaetes multiplication in the Vertebrate host is brought about by both longitudinal and transverse fission. Both processes have been repeatedly observed in life, and there has been no confusion of longitudinal division with either entanglement forms or the flexed or 'incurvation' form of transverse division, recently described by Gross (1910) for certain Spirochaetes of *Pecten jacobaeus*.

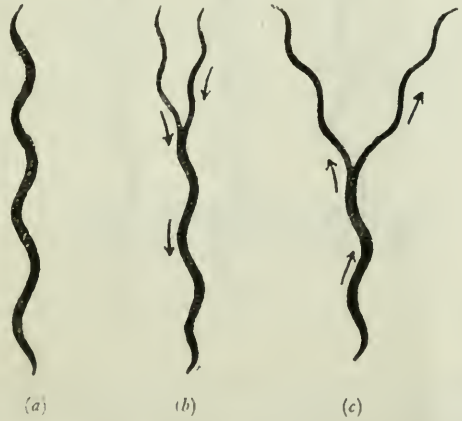

Fig 2.—Diagram illustrating longitudinal division. (*a*) Normal Spirochaete, waves passing alternately in either direction. (*b*) Waves passing from split to undivided end. (*c*) Return waves. Split extending.

True longitudinal division occurs in somewhat thicker individuals found at the beginning of infection and exhibiting a perfectly distinct

clear body, without any entanglement or curvature on themselves. Each organism may be in rapid backward and forward progression a second or so before the onset of division. Rapid waves of contraction followed by relaxation pass down the body of the Spirochaete. A split appears at one end, and gradually widens. The waves pass down each of the daughter forms, which diverge from one another (Fig. 2) until they lie at an angle of 180°, when separation occurs. At the commencement of longitudinal division by no means could a second body, or flexion of one body simulating such, be distinguished, no matter what form of examination were adopted. Longitudinal division is best observed at the onset of infection as recorded by Fantham and Porter in 1909. The resultant forms are half the width of, but the same length as, the body of the parent.

Balfour (1911*b*) has recorded longitudinal division in the Spirochaete (*S. granulosa*) of Sudanese fowls.

Transverse division, in my experience, occurs usually in straight forms. There is no need of looping, 'incurvation,' 'rolling up,'

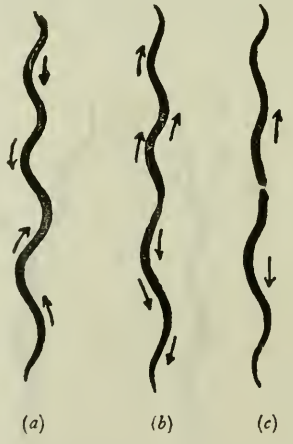

Fig. 3.—Diagram illustrating transverse division. (*a*) Shows waves passing from either end towards a centre or node. (*b*) Shows direction of return waves outwards. Node thinner as result of succession of waves outwards and inwards. (*c*) Daughter Spirochaetes moving away in direction of the outward waves.

or other contortion-figures as a preparation for the act of transverse division. Such contortion may occur, but my experience both with

these blood Spirochaetes and with those of Lamellibranchs is that such movements are but rarely preliminaries to division.

Ordinary straight-lying Spirochaetes, perhaps a little longer than their fellows, divide transversely. Waves pass from each end of the organism towards its centre, where they mutually extinguish one another and induce return waves towards the ends. These processes are repeated many times and the nodal point becomes somewhat thinner, while the newly forming organisms elongate slightly. By a final slight thinning of the node, separation is effected (Fig. 3).

'Delusion' and 'Contortion' division. At various times workers unable to observe longitudinal division of Spirochaetes, partly due to the periodicity of direction of division described by Fantham and Porter in 1909, have thought that the intertwining of two Spirochaetes and their subsequent separation has been mistaken for longitudinal division. Such is not the case. Large numbers of such interlocked forms have been observed and their significance fully realised. In such cases, at some period, two bodies are visible at the 'undivided' end. Further, the series of waves in intertwined forms is modified considerably, and the result is very unlike what is present in true longitudinal division.

Looped or flexed forms of Spirochaetes, which may divide transversely in some cases, have been suggested as possible explanations of longitudinal division. Here again, the two parts of the body of the flexed organism can be detected and also the flexion can be witnessed and recognised for what it is (Fig. 4).

Fig. 4.—Diagram of flexed and intertwined form. Transverse division may sometimes occur at the loop. Usually the organism uncurls and swims away. Alleged by some to be mistaken (!) for true longitudinal division.

Any one who has carefully watched Spirochaetes in life need fall into no such error regarding the nature of the movement. Such flexed or 'incurved' individuals may become slightly thinner and break at the point of flexion, but in my experience this is somewhat rare. The mode of division induced by wave motion has always been the usual method.

Transverse division occurs more particularly when the infection of Spirochaetes is abundant (Fantham and Porter, 1909) and hence is more easy of observation than is longitudinal division.

MULTIPLE TRANSVERSE FISSION OF THE BLOOD SPIROCHAETES WITHIN THE VERTEBRATE HOST

I have observed that a very small number of *S. duttoni*, *S. recurrentis* and *S. marchouxi*, while in the blood of their Vertebrate hosts pass through a peculiar form of asexual multiplication which, for want of a better term, I denote multiple transverse fission. The protoplasmic contents of the Spirochaete concentrate around the chromatin masses forming a number of segments within the periplast which acts as a sheath. A number of small, round or oval bodies ('granules') are thus formed. These may be the diameter of the body of the parent or, if they lie obliquely or curved as they sometimes do, may exceed it very slightly. The effect may be compared with a series of small biconvex or spherical tabloids within a thin skin. The individual small bodies may be compared with cocci. The periplast ultimately ruptures at one end and the small coccoid bodies, which I designate spores, issue into the blood stream (*cf.* Fig. 5, page 489).

In my opinion, this multiple transverse fission is scarcely an essential phase of the Spirochaete in the Vertebrate host, but may occur at the crisis, and may explain the 'after phase.' I regard the phenomenon largely as an anticipation of what occurs in the Invertebrate host, which is frequently a tick. Such resistant, sporular 'granules' may occur in or on the mammalian red cells in the case of *S. duttoni* or *S. recurrentis*, and may be seen sometimes apparently inside the avian red blood corpuscles in the case of *S. marchouxi*. Empty periplastic sheaths, from which the 'granules' have issued may sometimes be seen lying in the neighbourhood.

Balfour (1908) has stated that small ovoid bodies, or granules, formed by *Spirochaeta marchouxi* occur within the blood corpuscles. I have seen similar bodies on a few occasions. Prowazek (1906) recorded intra-corpuscular stages of *S. marchouxi*, and Breinl (1907) observed *S. duttoni* forming granules in the spleen.

I have also observed in some smears of human blood from a case which had apparently recovered from African tick fever, some

interesting intra-corpuscular forms of *S. duttoni*. The Spirochaetes appeared as spiral bodies with terminal swellings somewhat resembling spermatozoa. These Spirochaetes are like forms of *S. nicollei* (a variety of *S. marchouxi*) figured by Blanc (1911)* as occurring in the haemocoelic fluid of *Argas persicus*.

SOME OBSERVATIONS ON SPIROCHAETES (*S. DUTTONI, S. MARCHOUXI*) IN TICKS (*ORNITHODORUS MOUBATA*,† *ARGAS PERSICUS*†)

The foregoing is a brief account of the main results of my investigations of certain blood Spirochaetes in the Vertebrate host. With regard to stages of some of these organisms in the Invertebrate there is less definite information. Dutton and Todd, in 1905, showed that the tick *Ornithodorus moubata* was the carrier of *Spirochaeta duttoni*, and they saw the passage of the Spirochaete through the gut-wall into the body-cavity of the tick. They also demonstrated that hereditary infection of the ticks occurred, as did Koch. After an interval of some four years, Sir Wm. Leishman investigated further the exact method of transmission of *S. duttoni* by *O. moubata*. Since the early part of 1909 I have had the opportunity, both in Cambridge with Professor Nuttall, and in Liverpool, of carrying out some experiments confirming Leishman's work. Hindle (1911) has also recently confirmed the same. There is no need, then, for me to set forth my experiments in detail, but the results of laboratory experiments of mine may be briefly summarised as showing that infection of the salivary glands is not the common mode of infection (as was supposed by Koch), that the excretion from the Malpighian tubules of the tick is infective, and passes near the end of the period of feeding into the wound caused by the tick's bite; that within the adult tick the Spirochaetes undergo change, producing small forms. Some of the Spirochaetes in the intestine of the tick resist digestion therein to varying degrees. They may disappear as such in a few hours after the tick has fed; they usually disappear in a few (3 to 10) days, but may remain in the intestine, as

* It is to be regretted that in the earlier portions of Blanc's memoir (dealing with the structure, division and classification of Spirochaetes in general) the contents of several important memoirs are quite overlooked, although the papers in question are listed in his bibliography.

† The *Ornithodorus* came from Uganda, the *Argas* from Egypt.

Spirochaetes, for two or three weeks. This phenomenon partly depends on the temperature at which the tick is kept, 37° C. being an optimum for development of the Spirochaetes in the tick. Some Spirochaetes pass through the gut-wall of the tick and reach the haemocoelic cavity, where they may attach themselves to the colourless corpuscles floating in the haemocoelic fluid. The Spirochaetes then break up by multiple transverse fission into coccoid bodies (spores) composed of densely staining chromatin surrounded by a thin covering of cytoplasm, like those described in the blood. Certain Spirochaetes become intra-cellular in the gut-epithelium and alimentary diverticula, and may produce granules there. Ultimately some of the coccoid bodies reach the ovaries and ova, as well as the Malpighian tubules of the tick, where they may multiply within the cells of these organs.

STAGES IN THE TICK EMBRYO

As Leishman, Balfour, Hindle, and Blanc have recently published their observations in some detail it is quite unnecessary for me to recapitulate their work, consequently I will merely summarise my results.

It has been mentioned that Spirochaetes in the tick have the power of penetrating the gut-wall, reaching the body cavity and there in the haemocoelic fluid and its cellular elements forming minute ovoid or rod-like bodies. In the course of either the movement of the Spirochaetes or of the haemocoelic fluid, the ovoid bodies reach the ovary, where they intermingle with the developing ova, and become incorporated with some of them. The eggs when laid may contain these minute bodies. Recently laid eggs of *Ornithodorus* and *Argas*, crushed, made into an emulsion with a little sterile salt solution, and then inoculated into mice or chickens, were not often infective. On the other hand, when the eggs were kept in an incubator at 34° to 37° C. for four to six days before being injected, the experimental animals developed spirochaetosis and died in a short time (3 to 6 days). When bacillary forms had developed after keeping the eggs at 24° C., the contents of the crushed eggs*

* In experiments with eggs, the contents of 6 to 12 eggs were used each time.

were infective in three experiments in four to seven days. The results of my microscopical examination of tick eggs are as follows:—

1. Egg when laid shows no Spirochaetes. Extremely thin smears show a few ovoid bodies which are difficult of detection.

2. Egg three to five days incubated. The embryonic Malpighian tubes are developed. Some of the yolk is absorbed. The ovoid bodies can be more easily detected as groups in the Malpighian tubules. A few have begun to elongate.

3. An egg six to seven days incubated shows more organs of the tick formed. Many of the ovoid bodies have lengthened and become bacillary. At this stage they may rupture the cell in which they developed, and escape into the lumen of the Malpighian tubule.

4. Owing to development of organs it is difficult to follow the metamorphosis of bacillary or vibrio forms into fully formed Spirochaetes, but two methods seem possible (*a*) fusion of rods; (*b*) elongation and growth in thickness of bacillary forms. Probably the latter method chiefly occurs.

5. A recently hatched infective tick contains in its gut (*a*) ovoid bodies; (*b*) bacillary forms; (*c*) a very few fully developed Spirochaetes if kept at 35° C. for six or eight days.

STAGES IN TICK NYMPHS BORN OF INFECTED PARENTS

Nymphs of *Argas persicus* or of *Ornithodorus moubata*, born of infected parents, usually contain coccoid bodies (spores) and bacillary forms (which are elongating spores).

Experiments with such nymphs of *O. moubata*, kept at 24° C., show that they are capable of infecting mice with *S. duttoni* when fed on them. Three experiments positive.

Two experiments with two nymphs of *A. persicus*, born in the laboratory of infected parents, kept at 24° C. for six days, and fed on young pigeons, were negative. Similar results were obtained by Brumpt and by Blanc,* but my experiments are too few for generalisation as to the infectivity of nymphs of *Argas persicus*. Again, such nymphs may possess a natural immunity, or they may come from eggs which did not happen to become infected, or

* See Blanc (1911), p. 102. This author doubts the connection of 'granules' with the life-cycle of *S. nicollei* in *Argas*. See his 5th paragraph on p. 112.

possibly the nymphs required warming to a higher temperature (say 34° C.). I had no more nymphs for further experiments at the time.

SPIROCHAETES OF LAMELLIBRANCHS

Regarding the Spirochaetes of Lamellibranchs, I have already published several papers (1907, 1908, 1909) dealing with the morphology and division of these organisms, and my recent results merely confirm these. Naturally, morphological features can be elucidated in greater detail in the case of these larger organisms than in the case of the blood Spirochaetes. Membranes are easily seen in life and nuclear detail can be observed by the use of the paraboloid condenser, as has been shown by some previous workers (Porter, 1909). In this respect I must beg to differ from Gross (1910) in his view that Spirochaetes are enucleate.

With regard to division, both longitudinal and transverse division occur, and each is of the same type as seen in the blood Spirochaetes (*cf.* Figs. 2 and 3), and the same description applies. In transverse division the direct method without 'incurvation' appears to be common. The figures of 'incurvation' division published by Gross and others, are, in my opinion, far from convincing. Movements causing 'deception division' were described by Porter in 1909, and their significance stated. Recently, a revival of these rather old ideas has occurred, with the result that perfectly normal methods of longitudinal and transverse division have been overlooked, since attention was focussed on the rarer and possibly abnormal modes. I would direct attention to a paper by Porter (1909), in which a careful study was made of many peculiar movements of living Spirochaetes from Lamellibranchs. Also those Spirochaetes undergoing longitudinal division were again shown to be provided with two membranes, produced by the splitting of the original one, thus further confirming my work (1907-8-9). Borrel has also noted the occurrence of a double membrane in some specimens of *S. balbianii*. Further, double (that is, divided) chromatin granules can be seen, after staining, in Spirochaetes about to divide. Such have been figured by some authors who regard transverse division as the only mode (*cf.* Calkins, 1909, p. 221).

Spirochaeta balbianii of oysters, *Tapes* and other molluscs, *S. anodontae* and *S. solenis, n.sp.*, have been under my observation

for some years. During this time, small ovoid bodies ('spores') of the same diameter as the Spirochaetes have been found from time to time (*cf.* Fig. 5*e*) and also empty sheaths, in both the crystalline style, the intestinal contents and the water in which the Lamellibranchs were living. The significance of these forms was considered, but until the actual formation of them from Spirochaetes had been repeatedly seen (and confusion with extraneous bodies excluded), I did not wish to publish my results. Since cross-infection of the molluscs has been proved to occur by the Spirochaetes swimming out of the alimentary tract and mantle cavity of the mollusc into the surrounding water (Fantham, 1907-8), the production of spores has not so great a significance as in the case of the blood

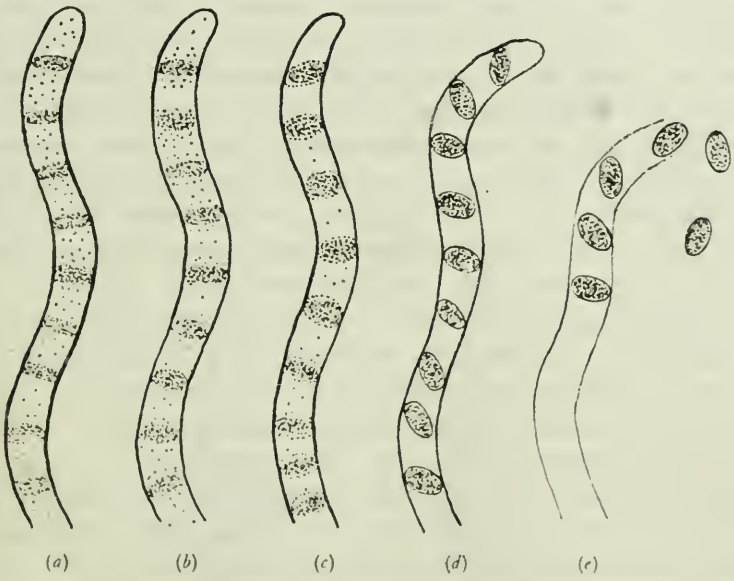

FIG. 5.—Shows formation of ovoid or coccoid bodies (as in *S. balbianii*) within part of Spirochaete. All details of membrane, etc., omitted for clearness' sake. (*a*) Normal form with chromatin bars. Tenuous cytoplasm. (*b*) Concentration of protoplasm round bars beginning. (*c*) Ovoid bodies differentiating. (*d*) Fully formed ovoid bodies within periplast. (*e*) Periplast ruptured and degenerating. Ovoid or coccoid bodies (spores) escaping.

forms. Bosanquet (1911) has also mentioned, in a recent note, the formation of coccoid bodies in a preparation containing *S. anodontae*. The method of formation of spores (Fig. 5 *a-e*) is identical with that seen in the blood Spirochaetes. I have seen spores issuing from *S. balbianii* and *S. anodontae* on several occasions (Fig. 5*e*).

The spores or coccoid bodies are probably able to withstand conditions unfavourable to the spirochaetiform stage of the parasite. Spores may also be formed in a fresh preparation of Spirochaetes kept moist for twenty-four hours. However, I do not consider that the coccoid bodies are products of degeneration, as degenerating Spirochaetes have a very different appearance.

Cross-infection by the agency of water has been shown. I have infected apparently clean *Tapes aureus* with *S. balbianii* by placing an infected oyster with them. Infected *Tapes* placed in water with a clean stock of *Tapes* result in all becoming infected. Similar experiments with *Ostrea edulis*, *Pecten jacobaeus** and *Tapes aureus* had the same result. *Sphaerium corneum* has been cross-infected with Spirochaetes from *Anodonta cygnea*, though with more difficulty. Water in the aquaria or basins in which infected individuals were placed has yielded Spirochaetes, and healthy molluscs introduced into this water have become infected. An intermediate host does not seem necessary for the transference of the Spirochaetes. Various commensals of oysters and anodons have been examined. *Atax bonzi*, from the mantle cavities of infected *Anodonta cygnea*, have been dissected, and in some of them, spores or bodies closely resembling them, have been found. Some of these bodies become rod-like; but as the complete metamorphosis of them into Spirochaetes has not been observed, it is well for the present, to consider them as under suspicion of being evolutionary stages of *S. anodontae*, though they might be separate bodies.

Previously, mention has been made of attempts to disprove longitudinal division by suggested 'explanations' that break down.

Much trouble has arisen in this and in other connections from a paper by Gross (1910) on the spirochaetal parasites of *Pecten*. Both Gross and those who have followed his lead, unfortunately show a regrettable lack of knowledge of the literature on the group, more particularly in connection with the subject of division. Had they noted carefully the paper by Miss Porter and myself (1909), and another dealing with movements simulating division by Porter (1909), it seems probable that they would not have assumed that transverse division following 'incurvation' had been mistaken for

* In consequence of infection experiments, I consider that *Cristispira pectinis* (Gross) is really *Spirochaeta balbianii*.

longitudinal fission, nor that the mechanism of division could be confused when there are such striking differences in movements.

Some writers have stated that Spirochaetes are homogeneous and undifferentiated in structure. The instructive results of compression of these organisms should be noted, and then the undoubted differentiation into periplast and protoplasm no longer presents difficulties. I have performed such experiments myself, and by crushing *S. balbianii*, the bars of chromatin issue intact with the cytoplasm and take the characteristic coloration on staining. The mode of formation of spores is a further indication of internal differentiation.

Encystment of Spirochaetes has been described by several writers. Up to the present, neither in the Spirochaetes of the blood nor in those of Lamellibranchs have I found true encystment. Two types of pseudo-cyst forms, have, however, been encountered:—

1. The Spirochaete becomes more closely coiled, either about its centre or nearer one end, so that a ball-like form is produced. This ball simulates a cyst with the body of the Spirochaete protruding

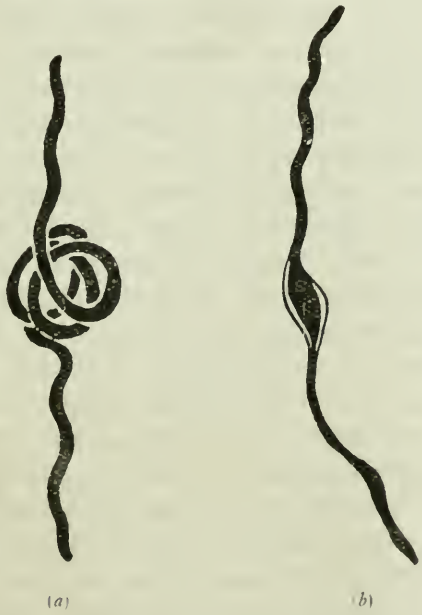

Fig. 6.—Pseudo-cysts. (*a*) Ball-like coil in centre of Spirochaete, which soon uncoils. (*b*) Plasmatic cyst or swelling.

from either end (Fig. 6*a*). But this form is only temporary, the Spirochaete uncoiling after a short time and swimming away normally.

2. Plasmatic cysts may be formed. Here the Spirochaete is dying, and so is not normal. The periplast relaxes and the cytoplasm tends to collect into small irregular masses or droplets, which cause local bulgings along the body. I have not seen these protoplasmic aggregations other than in animals in an almost moribund condition, when the Spirochaetes, naturally, were under unfavourable conditions (Fig. 6*b*). One or two similar plasmatic cysts may occasionally be found under similar conditions on a blood Spirochaete.

NOMENCLATURE

The re-naming of the Spirochaetes of Lamellibranchs by Gross (1910) as *Cristispira*, is, in my opinion, a mistake, is quite unnecessary, and should be disregarded. The Spirochaete group as a whole, (or various members of it), has received so many names that it seems to be a mania to re-name it according to individual fancy, as witness *Borrelia*, *Spiroschaudinnia*, *Spiroflagellata*, *Proflagellata*, *Spironemacea*, and now *Cristispira!*

The accounts of *Spirochaeta plicatilis*, the type species of the genus, given by Ehrenberg, by Schaudinn and by Zuelzer (1910) vary so much that they cannot be reconciled, and suggest that the authors may have dealt with different organisms. Zuelzer's work certainly needs confirmation. Hence, conclusions involving the structure of *S. plicatilis* cannot be accepted as a basis for changes in the nomenclature of the group. It should also be remembered that *S. plicatilis* is said to undergo multiple transverse fission, and in this respect resembles the Spirochaetes of Lamellibranchs and of the blood. It seems, then, that the new generic name *Cristispira* is unnecessary. It is much to be deplored that there are so many attempts at fresh classifications and nomenclature before the life-histories of the organisms are completely known.

The introduction of the term 'crista' for the membrane also is unnecessary. In 1907-8, I pointed out that there was a difference between the membrane of a Spirochaete and the undulating membrane of a Trypanosome, for in Spirochaetes the membrane

does not markedly undulate. (Fantham, 1908, pp. 31, 55, 58.) Hence, I referred constantly to the structure in Spirochaetes as a membrane only.

Quibbles as to whether bars or rings of granules of chromatin are present in the body of a Spirochaete, also speculations as to the exact shape of the organism need not have arisen, for the matter was discussed fully by me in 1908, and illustrated (text-fig. 6, p. 30) by drawings of sections of Spirochaetes cut *in situ* in the style of Anodon. I have since (1909) cut sections of infected styles of *Tapes aureus*, and have figured (Pl. VI, fig. 54) the sections of the Spirochaetes therein. Careful examination of these figures would be sufficient to settle such questions as those of shape of the body and disposition of the membrane to the satisfaction of any thinking person.

An interesting observation by Balfour (1911) may be noted here. He states that '*Treponema pallidum* is a granule shedder.' In that case, it may be that *T. pallidum* is really a member of the genus *Spirochaeta*, too minute for observation of a membrane or internal chromatin granules, and so its coils may only *appear* to be fixed. Further, the 'granules' of *Trep. pallidum* may explain the *Cytoryctes luis* of Siegel.

The numerous similarities between blood Spirochaetes and those of Lamellibranchs, then, justify the retention of them in the same genus. The morphology of their bodies, membranes and nuclei is similar. They divide by both longitudinal and transverse division. At one stage in their life-history they produce small, oval bodies within their periplasts. When liberated, these oval bodies serve either for re-infection of the same host or for cross-infection purposes. Hence, the life-history is on parallel lines.

SUMMARY

1. The Spirochaetes considered in this paper are *S. duttoni*, *S. recurrentis* and *S. marchouxi* (= *gallinarum*) among blood-inhabiting forms, also *S. balbianii* in *Ostrea edulis* and *Tapes aureus*, *S. anodontae* in *Anodonta cygnea* and *S. solenis** in *Solen ensis*. Both living and stained material have been used.

* *S. solenis* is about 40μ to 60μ in length in the specimens which I have measured. It is the salt-water counterpart of *S. anodontae*, both having pointed ends.

2. True longitudinal division, as well as transverse division has been observed in these Spirochaetes. There is a periodicity in the division of the blood-inhabiting Spirochaetes, transverse division occurring when the parasites are numerous in the blood, longitudinal division occurring at the beginning and end of infection.

3. Transverse division following flexion, or 'incurvation,' has been observed, but somewhat rarely. Transverse division usually occurs in relatively straight or unflexed forms. I do not consider that 'incurvation' is a necessary preliminary of transverse division.

Intertwined forms have not been mistaken for longitudinal division.

4. The protoplasmic contents of some of the Spirochaetes of the blood may break up into a number of small, round, or ovoid bodies, lying loose within the periplast, which ultimately ruptures at one end and sets them free. These minute bodies, variously known as 'coccoid bodies,' 'granules,' or 'spores,' are formed at the crisis. I doubt if these bodies represent an essential phase in the life-history of the Spirochaetes in the Vertebrate host, but are rather an anticipation of the similar phase in the Invertebrate hosts of these Spirochaetes. However, occasionally 'granules' may occur inside the red-blood cells.

5. Certain *S. duttoni*, when ingested by *Ornithodorus moubata*, and certain *S. gallinarum* ingested by *Argas persicus* pass through the intestinal wall of their hosts, and then form minute coccoid bodies, spores, or 'granules' by multiple transverse fission. Such granules, as well as Spirochaetes, may be found in the haemocoelic fluid of the ticks, in the Malpighian tubules and in the gonads.

6. Some of the Spirochaetes and spores reach the ovaries and ova of the infected parent tick. The spores concentrate in the Malpighian tubules of the developing embryo, which may be born infected.

7. Many nymphs of *O. moubata* born of infected parents are themselves capable of infecting. In the case of nymphs of *Argas persicus*, although various observers have recorded negative results, more experiments are necessary before it can be asserted that nymphs born of infected parents are themselves not infective.

8. The main source of infection from both adult and young ticks is the white excrement passed from the Malpighian tubules.

9. Elongation of the coccoid bodies, spores or 'granules' to

form short rods, and growth of these rods to form longer (or vibrio) forms has been observed in the tick. In this way young Spirochaetes are developed.

10. The Spirochaetes of Lamellibranchs do not necessarily depend on a carrier for change of Lamellibranch host. Cross-infection is brought about by water, which conveys not only active living Spirochaetes from the alimentary tract and mantle cavity of infected molluscs to the inhalent apertures of other molluscs, but also coccoid bodies (spores) may be thus conveyed and cross-infect. Coccoid bodies have been observed in process of formation in *S. balbianii* and *S. anodontae*. (Fig. 5.)

11. The life-cycle of the Spirochaetes of Lamellibranchs and of the Spirochaetes of the blood of Vertebrates follows a similar course. Their morphology is much the same, allowing for differences of size. There appears to be no justification for separating generically the Spirochaetes of Lamellibranchs from their allies in the blood of Vertebrates. (See p. 492.)

ADDENDUM

From a recent communication I gather that Frl. Dr. M. Zuelzer has in the press another paper on *Spirochaeta plicatilis*. Unfortunately, at the time of correcting proofs of this paper, Frl. Zuelzer's memoir is not published, and I am unaware of her conclusions. However, I should like to state, with all due respect to the various authors who have written on Spirochaetes, that it seems to me that much of the recent work on the group has been done by inexperienced investigators who, in consequence of their inexperience, are prone to make dogmatic and contradictory statements based on slender evidence, and have a penchant for putting forward new classifications. The literature on Spirochaetes at present is, in consequence, in a state of the utmost confusion. As one who has worked on many Spriochaetes in various parts of the world since 1906, I wish to state emphatically that I do not think serious attention should be paid to any work which does not set forth in clear and concise language the practically complete life-cycle of the organism under discussion, with clear and convincing reasons for any suggested new classification and appropriate illustrations of new features.

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